International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Rapid Green Synthesis of Metal Nanoparticles using Pomegranate Polyphenols

Abuelmagd M. Abdelmonem[a] & Rehab M. Amin[b*]

[a] *Food Technology Research Institute, Giza, 570020, Egypt.*

[b] *National Institute of Laser Enhanced Sciences, Cairo University, Giza, 12316, Egypt.*

[a] *a_almagd@yahoo.com*

[b] *rehabamin@niles.edu.eg*

Abstract

Plant extract could be an alternative to traditional chemical methods for the production of metallic nanomaterials in a clean, nontoxic and ecologically sound manner. In the present study, we aimed to develop a rapid ecofriendly method for the synthesis of both silver and gold nanoparticles using pomegranate peel extract (PPE). The bioactive constitutes and the potential antioxidant capacity of pomegranate (*Punica granatum* L.) extract seems to play a role in the production of metal nanoparticles. Biosynthesis of metal nanoparticles using PPE gives high yield of nanoparticles. The resulted nanoparticles provided high monodisperse character with an average particle size 50 nm for gold nanoparticles and 20 nm for silver nanoparticles. Moreover, rapid biosynthesis of silver nanoparticles occurred only at alkaline media. The production of nanoparticles by this method is extremely fast, ecofriendly and stable for several weeks with no additional surfactants.

Keywords: Nanoparticles; biosynthesis; plant extract; silver; gold; polyphenols.

1. Introduction

Metallic nanoparticles have been synthesized using different chemical methods, producing hazardous materials. Environmental issues of nanotechnology are becoming increasingly prominent area of research; therefore there is a need to synthesize nanoparticles that are safe and environmental friendly.

---

* Corresponding author.

E-mail address: rehabamin@niles.edu.eg

Biosynthesis of nanoparticles could be an alternative to traditional chemical methods for the production of metallic nanomaterials in a clean, nontoxic and ecologically sound manner [1-3].

Recently, biosynthesis of nanoparticles using plant extract has emerged an easy and viable alternative to traditional chemical and physical methods. Synthesis of nanoparticles using plants can provide more biocompatible nanoparticles than chemical synthesis. Whereas chemical synthesis may lead to the presence of some toxic chemical species on the surface of nanoparticles that may have undesirable effects in biomedical applications[4]. Several studies have demonstrated that biomolecules identified from biological organisms can be used to control the nucleation and development of the inorganic nano-structures[5]. Preparation of gold and silver nanoparticles by living plants were firstly reported by Gardea-Torresdey *et al.,* 1999[6]. The use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by reducing the complicated methods of maintaining cell cultures[7].Previously it was reported that plant extracts that contain different constitutes like polysaccharides, antioxidant metabolites, phenolic compounds and flavonoids [8-10] have been used for the biosynthesis of nanoparticles.

Pomegranate (*Punica granatum* L.) is a wide fruit that cultivated throughout the mediterranean regions. Numerous studies were reported on the antioxidant properties of pomegranate constituents[11]. It was reported that pomegranate contains some species of flavonoids and anthocyanidins, and shows potent antioxidant activity[12]. In particularly, the most essential constituents of pomegranate peel are phenolic compunds; gallic acid and other fatty acids; flavonols; flavones, flavanones; and anthocyanidins [11].

After all of the above, the question raised is whether pomegranate can reveal efficiency in the biosynthesis of metal nanoparticles? This question prompted us to investigate the effect of “pomegranate peel extract” in the biosynthesis of metal nanoparticles. However, the use of pomegranate peel extract for the biosynthesis of metal nanoparticles has not been reported yet. Therefore, in the current study, we evaluate the biosynthesis of both silver and gold nanoparticles from pomegranate pericarp extract.

2. Materials and Methods

2.1. Materials

All chemicals were used without further modification or purification. All solutions of reacting materials were prepared in deionized water prepared with a Milli-Q water purification system. All the glass containers were washed with aqua regia (HCl: HNO_3 = 3:1 (v/v)) and then with Milli-Q water. Double distilled (d.d) water was used in all experiments. Silver nitrate ($AgNO_3$), chlorauric acid ($HAuCl_4$) and sodium hydroxide were purchased from (Sigma Aldrich). A stock solution of tetrachloroauric acid (100 mM) were prepared by dissolving a carefully weighed quantity of $HAuCl_4$ in d.d water, in a conical flask. By the same way, a stock solution of silver nitrate (10 mM) was prepared.

2.2. Preparation of Pomegranate pericarps Extract

Thirty grams powder of dried pomegranate peel was soaked in 400 ml of deionized water. In this process, extraction is done by incubating the flask on an orbital shaker at the room temperature and agitating at 90 rpm for 24 hours. After the extraction, the aqueous solution was filtrated through Whatman filter paper number one. The reddish brown extract was kept in a dark bottle in the refrigerator at low temperature until use.

2.3. Biosynthesis of Metallic Nanoparticles

The biosynthesis reactions were performed (in volume of 10 ml) in sterile tubes containing double distilled water. Different volumes from the previously prepared pomegranate extract (30, 50,100, 150, 200&500µL) were added to the reaction tubes. The effect of pH was examined by adjusting the pH of the reaction tubes to different values 4, 7 and pH 10 using HCl and NaOH 1M solutions. Then 1ml from (10 mM) stock solutions of both tetrachloroauric acid and silver nitrate were added separately to the reaction tubes to obtain the final concentrations 1 mM.

2.4. Characterization of nanoparticles

The shape and size of the nanoparticles (Nps) were determined by transmission electron microscopy (TEM). Analyses were performed using a JEOL 2010F field-emission gun operating at 120 kV. A drop of highly diluted sample solution was deposited on an amorphous carbon-copper grid and left to evaporate at room temperature. For absorption measurements, aliquots (200 µl) of the resulting nanoparticles were transferred in 96-well plates, and absorptions were recorded within scan range of 350:750nm using UV-visible Power wave microplate reader spectroscopy (Biotech, USA). Data points represent mean values of at least three independent experiments.

3. Results & Discussion

In terms of the environmental concerns, green methods for the synthesis of nanoparticles are heightened to overcome the massive amount of hazards associated to the conventional methods. Biosynthesis of nanomaterials from plant extract offers a valuable contribution to nanobiotechnology. Recently, bio-reduction of metallic ions based on plant extracts are being attempted due to the ease of synthesis and greater stability of nanoparticles [2,13]. It was found that, by adding PPE to the aqueous solution of $HAuCl_4$, the color of the reaction mixture changed immediately from pale yellow to red. *Increase in the amount of added* PPE changed the color gradually to dark red.

Based on the absorption spectra, the biosynthesized AuNps with different values of pomegranate extract showed surface plasmon resonance (SPR) bands at about 540 nm *as shown in Figure 1.* These bands arise due to the surface plasmon-oscillation modes of conduction electrons, which are coupled, through the surface to external electromagnetic fields [14]. The single symmetrical SPR band suggested that the synthesized AuNps were almost spherical shape [15], which confirmed by the TEM image. Moreover, *the increase in the amount of added* PPE induces an *increase in the absorption intensity without any shift* in SPR.

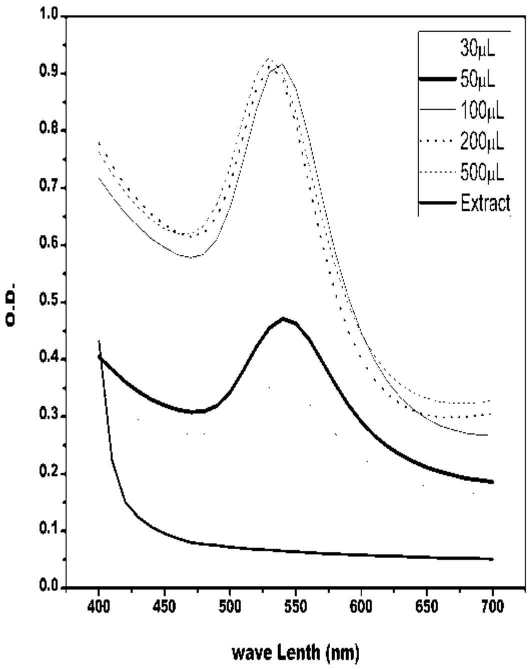


Fig. 1. UV-visible spectra of gold nanoparticles. Gold nanoparticles prepared from different values of pomegranate extract (30, 50, 100, 200,500µM).

TEM showed that the average particles size is 50 nm, (Figure 2,A) and the selected area electron diffraction pattern (Figure 2,B) indicated that the synthesized AuNps are single crystal. AuNps was observed immediately at neutral pH as reported previously [16]. By the same way, addition of PPE to the aqueous solution of silver nitrate, changed the color of the reaction mixture from pale yellow to brown, indicating the formation of AgNps [8,17].

The synthesized AgNps show a single SPR at 410 nm (*Figure 3*). TEM of the synthesized AgNps showed average particles size at 20 nm, (Figure 4,A) and the selected area electron diffraction pattern (Figure 4,B) showed that the synthesized AgNps are single crystal. *It was observed that* AgNps could be synthesized only *at pH (10.5), whereas* the reducing ability of bio-molecules is significantly increased at alkaline condition [18]. In absence of the hydroxide ion, the time taken for reduction of Ag^+ ions was longer[19,20].

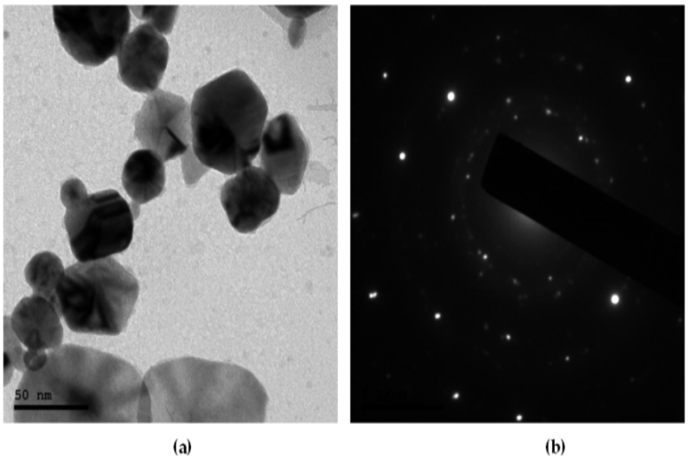


Fig**.** 2. Transmission electron microscopy (TEM) images of gold nanoparticles. (a) ; TEM of the biosynthesized gold nanoparticles & (b); selected areas electron diffraction pattern corresponding to a. Gold nanoparticles were prepared from pomegranate extract (100μM) with $HAuCl_4$ concentrations 1mM.

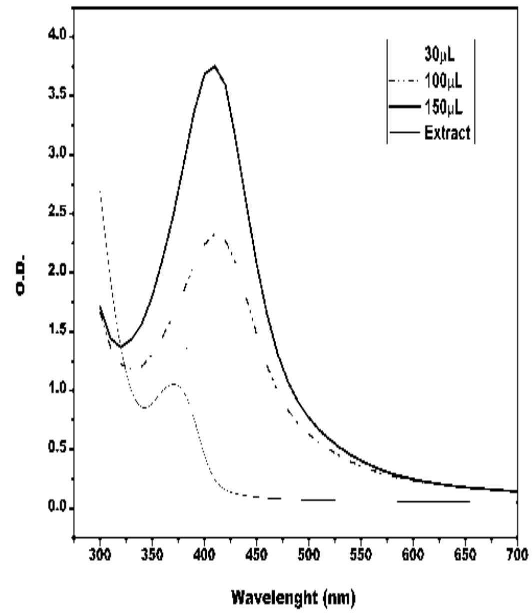
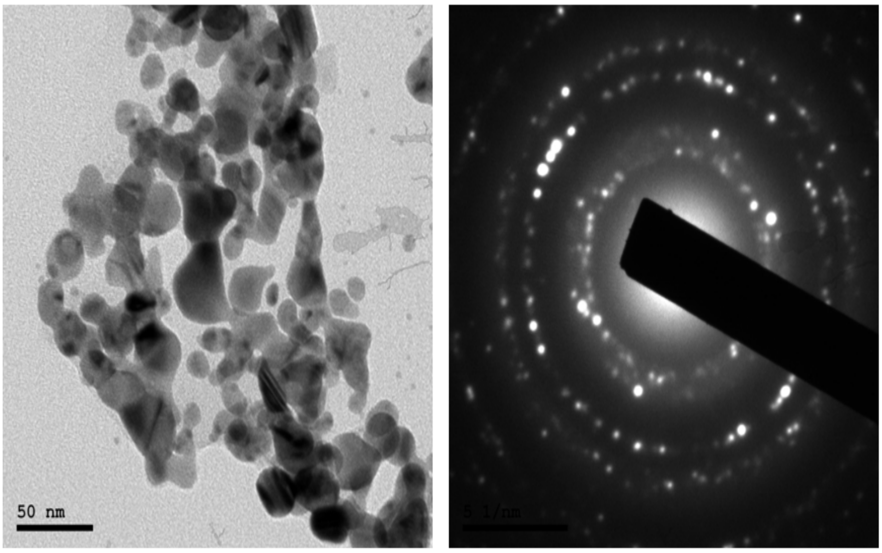


Fig. 3. UV-visible spectra of silver nanoparticles. Silver nanoparticles prepared from different values of pomegranate extract (30, 100, 150μM).



(a) (b)

Fig. 4. Transmission electron microscopy (TEM) images of silver nanoparticles. a; TEM of the biosynthesized silver nanoparticles & b; selected areas electron diffraction pattern corresponding to a. Silver nanoparticles were prepared from pomegranate extract (150µM).

At the time being, mechanism leading to the biosynthesis of metal nanoparticles is not yet fully understood. However, phenolic compounds that considered as one of the most essential constituents in PPE [11,21] could play a vital role in the reduction of metal ions (M^+) to metal nanoparticles (M^0) [2,22].

The resulted nanoparticles were stable for several weeks without addition of any surfactants. This stability could be due to the presence of flavonoids in PPE [11,12,23], which could cover the surface of nanoparticles. It was stated that, flavonoids in plant extract are surface active molecules that play a vital role in covering or capping the surface of metal nanoparticles [24,25].

In general, by increasing the amount of added PPE, the intensity of SPR of the resulted metal nanoparticles increased. The highest value of the biosynthesized AuNps was obtained after addition of

100µL PPE (Figure 1) while that for AgNps was 150µL (Figure 3). In addition, no more increase in intensity of SPR was observed even by increasing the amount of added PPE.

4. Conclusion

The production of metallic nanoparticles from pomegranate extract is a rapid, easy and clean way providing an innovative methodology to Nanobiotechnology. Nanoparticles synthesized by this process were stable for several weeks. Therefore, biosynthesis of metal nanoparticles using pomegranate extract will be a better alternative method to traditional, physical, and chemical and even the microbial synthesis.

5. References

[1] A. Mondal, S. Mondal, S. Samanta, and S. Mallick, "Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex used as an Important Taxonomic Tool for Phylogenetic Inter-relationship," *Adv. Biores.*, vol. 2, no. 1, pp. 122–133, 2011.

[2] S. Ghosh, S. Patil, M. Ahire, R. Kitture, S. Kale, K. Pardesi, S. S. Cameotra, J. Bellare, D. D. Dhavale, A. Jabgunde, and B. A. Chopade, "Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents," *Int. J. Nanomed.*, vol. 7, pp. 483–496, 2012.

[3] J. Kasthuri, K. Kathiravan, and N. Rajendiran, "Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach," *J. Nanopart. Res.*, vol. 11, no. 5, pp. 1075–1085, Sep. 2008.

[4] N. Ahmad, S. Sharma, V. N. Singh, S. F. Shamsi, A. Fatma, and B. R. Mehta, "Biosynthesis of Silver Nanoparticles from Desmodium triflorum: A Novel Approach Towards Weed Utilization.," *Biotech. Res. Int.*, Jan. 2011.

[5] E. Ruiz-Hitzky, M. Darder, and P. Aranda, "An Introduction to Bio-nanohybrid Materials," in *Bio-inorganic Hybrid Nanomaterials*, L. Y. Ruiz-Hitzky E., Ariga K., Ed. John Wiley & Sons., 2008, pp. 1–40.

[6] J. Gardea-Torresdey and K. Tiemann, "Gold nanoparticles obtained by bio-precipitation from gold (III) solutions," *J. Nanopart. Res.*, vol. 1, pp. 397–404, 1999.

[7] J. M. R. K. Sahayaraj, S. Rajesh, "Silver nanoparticles biosynthesis using marine alga padina pavonica (linn.) and its microbicidal activity," *Dig. J. Nanomat. Bio.*, vol. 7, no. 4, pp. 1557–1567, 2012.

[8] S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, and S. Thangamani, “Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities,” *Asian Pacific J.Trop.Biomed.*, vol. 2, no. 7, pp. 574–580, Jul. 2012.

[9] T. Liang, W. Yue, and Q. Li, “Comparison of the Phenolic Content and Antioxidant Activities of Apocynum venetum L. (Luo-Bu-Ma) and Two of Its Alternative Species,” *Int. J. Mol. Sci.*, vol. 11, no. 11, pp. 4452–4464, 2010.

[10] K. Satyavani, S. Gurudeeban, T. Ramanathan, and T. Balasubramanian, “Biomedical potential of silver nanoparticles synthesized from calli cells of Citrullus colocynthis (L.) Schrad.,” *J.Nanobiotech.*, vol. 9, no. 43, 2011.

[11] J. S. Jurenka, “Therapeutic applications of pomegranate (Punica granatum L.): a review.,” *Alter. Med. Rev.*, vol. 13, no. 2, pp. 128–144, Jun. 2008.

[12] J. Mori-Okamoto, Y. Otawara-Hamamoto, H. Yamato, and H. Yoshimura, “Pomegranate extract improves a depressive state and bone properties in menopausal syndrome model ovariectomized mice.,” *J. Ethnopharmacol.*, vol. 92, no. 1, pp. 93–101, May 2004.

[13] D. Jain, H. K. Daima, S. Kachhwaha, and S. L. Kothari, “Synthesis of Plant-Mediated Silver Nanoparticles Using Dioscorea batatas Rhizome Extract and Evaluation of Their Antimicrobial Activities,” *J. Nanomater.*, vol. 4, no. 3, pp. 557 – 563, 2009.

[14] A. L. Stepanov, J. R. Krenn, H. Ditlbacher, A. Hohenau, A. Drezet, B. Steinberger, A. Leitner, and F. R. Aussenegg, “Quantitative analysis of surface plasmon interaction with silver nanoparticles.,” *Opt. Lett.*, vol. 30, no. 12, pp. 1524–6, 2005.

[15] Z. Guo, Y. Zhang, A. Xu, M. Wang, L. Huang, K. Xu, and N. Gu, “Layered Assemblies of Single Crystal Gold Nanoplates: Direct Room Temperature Synthesis and Mechanistic Study,” *J. Phys. Chem. C*, vol. 112, no. 33, pp. 12638–12645, Aug. 2008.

[16] S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang, and N. Gu, “Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata,” *Mater. Lett.*, vol. 61, no. 18, pp. 3984–3987, Jul. 2007.

[17] S. Arulkumar and M. Sabesan, “Rapid preparation process of antiparkinsonian drug Mucuna pruriens silver nanoparticle by bioreduction and their characterization,” *Pharm. Res.*, vol. 2, no. 4, pp. 233–236, 2010.

[18] S. Gurunathan, K. Kalishwaralal, R. Vaidyanathan, D. Venkataraman, S. R. K. Pandian, J. Muniyandi, N. Hariharan, and S. H. Eom, “Biosynthesis, purification and

characterization of silver nanoparticles using Escherichia coli.," *Colloids Surf. B*, vol. 74, no. 1, pp. 328–335, 2009.

[19] Y. Feng, Y. Yu, Y. Wang, and X. Lin, "Biosorption and bioreduction of trivalent aurum by photosynthetic bacteria Rhodobacter capsulatus.," *Curr. Microbiol.*, vol. 55, no. 5, pp. 402–408, 2007.

[20] T. L. Riddin, M. Gericke, and C. G. Whiteley, "Analysis of the inter- and extracellular formation of platinum nanoparticles by Fusarium oxysporum f. sp. lycopersici using response surface methodology.," *Nanotech.*, vol. 17, no. 14, pp. 3482–3489, 2006.

[21] G. Miguel, S. Dandlen, D. Antunes, A. Neves, and D. Martins, "The Effect of Two Methods of Pomegranate (Punica granatum L) Juice Extraction on Quality During Storage at 4°C," *J. Biomed. Biotechnol.*, no. 5, pp. 332–337, 2004.

[22] J. Xie, Y. Tan, and J. Lee, "Biological and biomimetic synthesis of metal nanomaterials," in *Nanomaterial for Life Science*, vol. 7, C. Kumar, Ed. 2010, pp. 251–281.

[23] M. Miguel, "Pomegranate (Punica granatum L.): A medicinal plant with myriad biological properties-A short review," *J. Med. Plan. Res.*, vol. 4, no. 25, pp. 2836–2847, 2010.

[24] M. Dubey, S. Bhadauria, and B. Kushwah, "Green synthesis of nanosilver particles from extract of Eucalyptus hybrida (safeda) leaf," *Dig. J. Nanomater. Bios.*, vol. 4, no. 3, pp. 537–543, 2009.

[25] N. Saeed, M. R. Khan, and M. Shabbir, "Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L.," *BMC Complem. Alter. Med.*, vol. 12, no. 1, p. 221, Jan. 2012.